Prev ntion and tr atm nt of s psis and septic shock

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Most of the recent publications on the therapy or prevention of sepsis and septic shock have reported on attempts to interfere with inflammatory processes. Several approaches, aimed at inhibiting tumour necrosis factor or IL-1, have now been tested in phase II or III clinical trials, often with negative or doubtful results, but such trials only had the power to detect benefits of large magnitude. Many lessons can, however, be drawn from these disappointing data, and will perhaps lead to a better understanding of the pathophysiology of the disease, particularly regarding its complexity and the potential for practical applications in the future.

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Abbreviation

TNF tumour necrosis factor

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Introduction

Adequate antimicrobial therapy is still the cornerstone in the treatment of sepsis and septic shock, as is surgery when required. During the past three decades, new antibiotics have become available, which show potent activity against most bacteria. The most recent development in this field is the growing evidence that most patients with sepsis can be treated with single antibiotics such as carbapenem or third generation cephalosporins. Indeed, several studies [1,2] have shown that this kind of monotherapy is as effective and is less toxic than standard regimens associating a beta-lactam with an aminoglycoside.

Despite these advances and other progress in supportive care for organ failures, the overall mortality of septic patients remains approximately 40%, reaching about 70% in patients with established shock [3•]. In addition, the efficacy of antibiotics is being challenged by the rising incidence of infections which are caused by resistant pathogens. These developments have motivated a great endeavour aimed at achieving a better understanding of the pathophysiology of the inflammatory response to infection, and at the identification of new therapeutic targets. Most recent publications deal with these new approaches. Few domains of medical science have aroused as much hope as the immunotherapy of sepsis and septic shock, and few have seemed closer to achieving a successful link between basic research and clinical application. In the past 3 years, however, disappointing results have followed one another, and the bitterness of disillusion has dashed many of these hopes. The past year would appear to be the 'trough of the wave', because the bad news seems to be of much greater significance than the scarce optimistic data.

Bad news in immunotherapy

In 1991, two monoclonal antibodies, called HA-1A and E5, were reported to be beneficial in patients with infections caused by Gram-negative organisms [4,5]. Both antibodies were directed against lipid A, which is the well conserved toxic part of the endotoxin on Gram-negative bacteria. The results of these two trials generated an important controversy regarding their statistical validity and the unconvincing data on the actual neutralizing activity of the antibodies [6]. A follow-up study [7] that failed to confirm the efficacy of HA-1A has already been reported. Bone *et al.* [8] published a follow-up study with E5 in 847 patients that also turned out negative results. E5 was shown to be unable to reduce the 30-day mortality in the group of patients thought to benefit from it, that is in non-shock patients with Gram-negative sepsis. The failure of

these trials concluded three decades of research on anti-endotoxin approaches, although the concept of anti-lipid A antibodies is still theoretically valid. ??

More recently, the inhibition of several pro-inflammatory cytokines has been considered to be a potential target of prevention or therapy. The mounting evidence that cytokines such as tumour necrosis factor (TNF)-α and IL-1 are involved in the pathophysiology of sepsis caused by both Gram-negative and Gram-positive organisms make them even more interesting targets than endotoxin [9]. So far, however, most clinical trials have yielded negative results. In 1994, a double-blind, phase III study [10] of an antagonist of the IL-1 receptor showed no benefit on 28-day survival compared with placebo in 893 patients with a systemic inflammatory response syndrome. Further development of the drug has recently ceased. With regard to monoclonal antibodies directed against TNF-α, the results of three trials [11•–13•] have been published during the past year. None of the antibodies tested was able to reduce the overall 28-day mortality. In the first study [11•], 122 patients were randomly assigned to receive one of three different open label doses of the anti-TNF-α antibody MAK 195F, or placebo. A retrospective analysis suggested that there was benefit in a subgroup of patients who had an IL-6 level of more than 1000 pg/ml at the time of selection, and a further trial is now in progress. In the Bayx1351 trial (the NORASEPT trial) [12•], patients with sepsis syndrome were randomly assigned to receive either 7.5 or 15 mg of anti-TNF-α monoclonal antibody, or placebo. Interim analysis among the non-shock patients showed that a slightly higher mortality rate was observed in patients receiving anti-TNF-α antibody. Enrolment in the non-shock arm of the study was therefore discontinued. The final enrolment was 971 patients, with 49% in shock at entry to the study. There was no difference in all-cause mortality at 28 days in patients receiving placebo or antibody. Neither was there any difference among patients with septic shock, but a trend towards a reduction in mortality was observed at 3 days (15 mg/kg: 44% reduction versus placebo, $P=0.01$; 7.5 mg/kg: 49% reduction, $P=0.004$). In contrast, there was a non-statistically significant trend towards increased mortality in non-shock patients who received 15 mg/kg anti-TNF-α antibody. In the INTERSEPT trial with the Bayx1351 antibody [13•], there was a statistically significant effect on shock reversal and the development of organ/system failure, although this was not reflected in an overall survival benefit. It must be borne in mind that these studies were designed as phase II trials, and therefore had an 80% power to detect a reduction of mortality of large magnitude only (more than 50%), because of comparisons between multiple groups or sub-group analysis.

As well as by the use of monoclonal antibodies, neutralization of TNF can be achieved by perfusing a fusion protein that is a soluble receptor ligated to the Fc piece of an IgG molecule to improve its pharmacokinetics. Such a construct aims at diverting TNF from its cellular targets [14]. In the first available trial using this approach [15•], 141 patients with septic shock were randomly assigned to receive either placebo or escalating doses of a construct made up of the soluble, extracellular portion of the p75 TNF-receptor and the Fc piece (p75TNFR:Fc). The construct was not effective in preventing death at 28 days. Moreover, increasing doses were associated with increased mortality. Although an imbalance between groups cannot be ruled out because of the small sample size, these unexpected results have given rise to the hypothesis that this construct might have functioned as an intravascular carrier of TNF, prolonging the inflammatory response. Evidence for this hypothesis is shown in the difference between the results obtained in animal models with both existing fusion proteins. Indeed, there is another construct that bears the second type of TNF-receptor (p55TNFR), and the binding of this p55TNFR:Fc construct to TNF is more stable than the binding of p75TNFR:Fc. Evans *et al.* [16] were able to show a protective effect with p55TNFR:Fc, but not with p75TNFR:Fc in a model of Gram-negative sepsis. It is also possible, however, that the complete removal of TNF might be harmful, at least for a subgroup of septic patients.

Little good news in immunotherapy

A very similar approach has been used in another trial [17], but the extracellular portion of the p55TNF-receptor, instead of p75, has been chosen to be ligated to the Fc portion of the IgG. In a phase II, double-blind, randomized study [17], 498 patients have been assigned to receive either placebo or three different doses of the construct. Although no efficacy in preventing death could be found in patients prospectively defined as suffering from septic shock, a 36% reduction in mortality was noted in patients with severe sepsis but no shock who had receive the highest dose of the construct. The difference between these results and those of the p75TNFR:Fc trial may have arisen from the selection of patients with a less severe disease. It could also be caused by the fact that TNF is more stably linked to its p55 receptor than to p75, and is then less likely to be slowly released. A phase III trial is now in progress in patients with severe sepsis.

Reasons for hope

As stated in several editorials last year [3,18•,19], many lessons can be drawn from all these discouraging results, and from the ongoing research, which may contribute to future progress in the field.

Design of clinical trials and selection of patients

Up to now, the survival after 28 days has been chosen as the main outcome of all available trials. This may not, however, be sensitive enough to detect a beneficial effect of a new drug. Indeed, a reduction of mortality will perhaps only be achieved in a given subset of patients, or by

combining several innovative approaches. In this respect, some variables such as the time to occurrence or resolution of organ failures may become of interest to select a new compound for further evaluation. The adequacy of antibiotic or surgical therapy remains the most important therapeutic predictor of outcome. Future trials will have to take care not to be confounded by an imbalance of this important covariate. An example of such an imbalance was recently given by Sprung *et al.* [20], who performed a blinded clinical evaluation of the INTERSEPT trial, looking for confounding factors that interfered with the potential of a new drug to exercise its protective effect. They found that the proportion of patients with confounding events was greater in the high-dose anti-TNF treatment arm than in the two other arms of the trial.

Finally, the selection of patients will become a key issue. Most often, the clinical trials used the classification of the systemic inflammatory response to infection into sepsis, severe sepsis and septic shock [21]. This classification is still controversial, however, because it lumps together infections that differ in their causative organisms, the organs that are involved, their mortality rates, and probably their pathophysiology. For example, the clinical course of infections such as urosepsis, nosocomial pneumonia or fulminant meningococcemia illustrates how inhomogeneous each arm of a trial can be.

Several new markers of infection are currently under investigation to improve the delineation of subgroups of patients. For example, several papers have recently confirmed that procalcitonin may become a useful tool to distinguish bacterial infections from viral infections or from non-infectious inflammation in critically ill patients [22–24]. Pilz *et al.* [25] reported on the prognostic value of the soluble TNF-receptor p55 to predict the evolution of the APACHE II score in patients with a high initial score after surgery. Unfortunately, these results do not have practical implications because of the small sample size and the lack of information on infections in these patients. As already mentioned, high serum levels of IL-6 at baseline appeared to predict a dose-dependent reduction of mortality on day 14 in patients receiving the MAK 195F anti-TNF-α antibody [11•]. This retrospective analysis of a small subgroup of 37 patients is only an hypothesis that is currently being tested in an ongoing trial. Another topic of interest is the genomic polymorphism within the TNF locus. Stuber *et al.* [26] have characterized a bi-allelic locus in 40 patients with severe sepsis in a surgical intensive care unit. They found that the most frequent of these alleles was correlated with a greater TNF release and a higher mortality in homozygotous patients.

Progress in understanding pathophysiology

It might well be that we will not be able to achieve therapeutic or preventative goals in septic patients by inhibiting isolated endogenous mediators of the inflammatory response, such as TNF-α or IL-1. Our understanding of the pathophysiology is moving towards the recognition of a complex interplay of a large number of mediators that have either pro- or anti-inflammatory effects. It may be that the development of severe infection into septic shock or not depends on whether pro- or anti-inflammatory pathways are activated [18•]. The complexity of the cytokine network is illustrated by recent experiments with IL-10, a cytokine known for its inhibitory effect on monocytes [27]. Using IL-10-deficient mice, Berg *et al.* [28] were able to demonstrate a relevant beneficial role of IL-10 in a model of endotoxaemia, although this is mostly a TNF-dependent model. This result has been confirmed by Standiford *et al.* [29]. IL-10 was also shown to reduce mortality from peritonitis caused by caecal ligation and puncture [30]. Its anti-inflammatory effect, however, proved to be harmful in models such as infection with *Listeria monocytogenes* [31] or bacterial pneumonia [32,33].

The understanding of the complex cytokine pathways during infection will perhaps have therapeutic implications, such as the definition of subpopulations of patients who could benefit from a given therapy, or the development of trials of combined therapy directed against several mediators. This last approach deserves careful evaluation, however, as recently illustrated by observations from Opal *et al.* [34]. These authors used a model of lethal *Pseudomonas aeruginosa* infection in neutropenic rats in which the inhibition of TNF by a recombinant soluble TNF-receptor, or the inhibition of IL-1 by a recombinant receptor antagonist, afforded a protection of 31 or 30%, respectively. All the animals receiving a combination of both inhibitors, however, died with disseminated microabcesses, again emphasizing the potentially harmful effect of interfering with inflammatory processes.

A growing amount of data is also becoming available on molecules that may be new important targets for prevention or therapy in the near future. These include nitric oxide [35], lipopolysaccharide-binding protein [36], the bactericidal/permeability increasing protein [37,38], the CD14 receptor and its soluble form [39], E-selectin [40], circulating lipoproteins [41], analogues of lipid A [42,43] and the macrophage migration inhibitory factor [44•]; a non-exhaustive list. Also of interest is the demonstration by Echtenacher *et al.* [45•] of the protective role of mast cells and mast cell-derived TNF in acute polymicrobial peritonitis. All these new candidates will need to be evaluated with caution, bearing in mind what has been learnt from previous attempts.

Conclusion

From the development of the drugs that have been used in phase II or III studies, we have learnt that the gap between the animal models and the clinical application is wider than initially thought. It is probably unrealistic to look for one animal model that fits all the cases of sepsis in humans. On

the other hand, we have not yet been able to define a subgroup of patients in which the dramatic results from animal experiments could be applied. We are still, however, exploring the fine regulation of very potent mechanisms, which we hope may possibly lead to preventive and therapeutic applications.

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